AssayMax™

Human Ceruloplasmin ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 25 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
1								
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Human Ceruloplasmin ELISA Kit

Catalog No. EC4101-1

Sample insert for reference use only

Introduction

Ceruloplasmin is an abundant alpha-2-serum glycoprotein that contains 95% of the copper found in the plasma of vertebrate species (1). Ceruloplasmin is a copper-binding protein that normally removes iron from cells by its ferroxidase activity. Ceruloplasmin concentration on average is 14.6 (±4.0) mg/dl (2). Low levels of ceruloplasmin lead to the abnormal deposition of iron in cells, including those of the pancreas, liver, retina, and the basal ganglia region of the brain (1, 3-5).

Principle of the Assay

The AssayMax Human Ceruloplasmin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of ceruloplasmin in human **urine, saliva, milk, CSF, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures ceruloplasmin in less than 4 hours. A polyclonal antibody specific for ceruloplasmin has been pre-coated onto a 96-well microplate with removable strips. Ceruloplasmin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for ceruloplasmin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human Ceruloplasmin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human ceruloplasmin.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Ceruloplasmin Standard:** Human ceruloplasmin in a buffered protein base (960 ng, lyophilized).
- **Biotinylated Human Ceruloplasmin Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against human ceruloplasmin (140 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:10 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:16 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:1000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:400 into MIX Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Sample Dilution Guidelines below for further instruction.

Guidelines for Dilutions of 1:100 or Greater
(*for reference only; please follow the insert for specific dilution suggested)*

1:100	**1:10000**
A) 4 ul sample: 396 µl buffer(100x) = 100 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1:1000	**1:100000**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.

- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 960 ng of Human Ceruloplasmin Standard with 3 ml of MIX Diluent to generate a 320 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (320 ng/ml) 1:4 with MIX Diluent to produce 80, 20, 5, and 1.25 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 14 days.

Standard Point	Dilution	[Ceruloplasmin] (ng/ml)
P1	1 part Standard (320 ng/ml)	320.0
P2	1 part P1 + 3 parts MIX Diluent	80.00
P3	1 part P2 + 3 parts MIX Diluent	20.00
P4	1 part P3 + 3 parts MIX Diluent	5.000
P5	1 part P4 + 3 parts MIX Diluent	1.250
P6	MIX Diluent	0.000

- **Biotinylated Human Ceruloplasmin Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human Ceruloplasmin Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

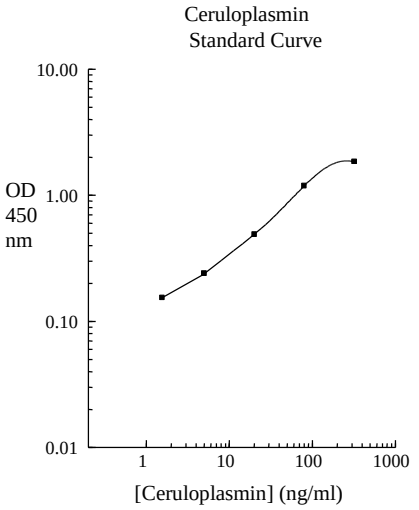
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human Ceruloplasmin Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 15 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of ceruloplasmin is typically ~ 1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.1% and 7.1% respectively.

Linearity

Average Percentage of Expected Value (%)	
Sample Dilution	Milk
1:500	89%
1:1000	96%
1:2000	99%

Recovery

Standard Added Value	1.25 – 80 ng/ml
Recovery %	85 – 116%
Average Recovery %	98.5%

Cross-Reactivity

Species	Cross Reactivity (%)
Beagle	None
Bovine	None
Monkey	<70%
Mouse	None
Rat	None
Swine	<10%
Rabbit	None
Human	100%

References

(1) Harris, Z. L, *et al*. *Proc. Natl. Acad. Sci.* Vol. 92, pp. 2539-2543, March 1995
(2) Aliyazicioğlu, Y *et al*. *The Turkish Journal of Pediatics* 2007; 49: 52-54
(3) Czaja, M. J *et al*. *J. Clin. Invest.* Volume 80, October 1987, 1200-1204
(4) Kumar, A *et al*. *WJM, October 1995-Vol 163, No. 4*
(5) Moller, L.B. *et al*. *Am. J. Hum. Genet. 66:1211–1220, 2000*

Version 2.0R

Related Products

- EC4001-1 AssayMax Human Ceruloplasmin ELISA Kit (Plasma and Serum samples)
- ERC4101-1 AssayMax Rat Ceruloplasmin ELISA Kit (Urine and Cell Culture samples)
- ERC4001-1 AssayMax Rat Ceruloplasmin ELISA Kit (Plasma and Serum samples)